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Effects of soil fertility and cotton rhizosphere on populations of Collembola

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With one figure

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1. Introduction

Rhizosphere ecology in relation to plant growth and root diseases has become a subject of renewed interest in recent years. Rhizosphere research has been reviewed and analyzed by CLARK (1949), STARKE (1958), KATZNELSON (1965), ROVIR and DAVEY (1974) and BOWEN and ROVIRA (1976). That the nutritional components of root exudates provide the stimulus for increased microfloral activity at the root surface has been well established. KEVAN (1965) discussed the nature and biology of the soil fauna in relation to the microflora; yet, with the exception of nematodes and protozoa, the fauna within this unique zone of root influence has been largely neglected.

It is general knowledge that numbers of soil microarthropods vary under different types of plant cover. Pasture crops with dense root systems generally can be expected to yield larger populations than cultivated row crops, thus reflecting a root influence. Since many species of Collembola feed on fungi and bacteria (CHRISTIANSEN 1964, ANDERSON and HEALEY 1972, CHRISTEN 1974), and the animals may produce higher populations in presence of the microflora (FARAHAT 1966, WIGGINS 1976), it is logical to expect that the rhizosphere may influence collembolan activities. Many actual and potential interactions between the microflora and fauna are revealed in the reviews of CHRISTIANSEN (1964); BUND (1970); BUTCHER, SNIDER, and SNIDER (1971) and CHRISTEN (1974). Altered soil fertility by use of inorganic fertilizers influences both plant growth and the quality and quantity of the microflora (SADASIVAN 1965, CURL and RODRIGUEZ-KABANA 1974). BLAIR (1978) showed that nitrogen in particular was important in determining the R/S (rhizosphere-to-nonrhizosphere) value for fungi and bacteria in seedling cotton. WALLWORK (1976) has discussed some effects of fertilization and other agricultural practices on microarthropods in general and provided pertinent references. Increased populations of soil Collembola in response to both organic and inorganic fertilizers were demonstrated by EDWARDS and LOFTY (1969).

The present study was conducted to determine the influence of cotton (*Gossypium hirsutum* L.) rhizosphere in fertile and nonfertile soil on populations of the predominant genera of Collembola, which would be used later in interaction studies.

2. Materials and methods

Sampling of both rhizosphere and nonrhizosphere soil was performed with a conventional soil-core sampling tube, 2.2 cm inside diam. A larger tube could not be used because of the desirability of obtaining as much true rhizosphere soil as possible, and a smaller tube might have caused excessive damage to the animals. The sampling tube was inserted against the plant stem and parallel to the tap root (Figure 1) to a depth of 20—25 cm, and the sample was quickly and gently ejected into a plastic pail; thus the tube samples (ca. 300/0.4 ha) were composited but not further mixed. Non-

Fig. 1. Method of collecting rhizosphere soil from cotton plants with a conventional soil-sampling tube.

rhizosphere samples were taken about 20 cm distant from the tap root zone. The conventional method of taking rhizosphere samples, by lifting plants and shaking or washing off only the soil adhering to roots, could not be employed for collembolans because many would escape or be damaged. Thus, each core sample contained some nonrhizosphere soil but also contained numerous secondary root fragments; therefore, the term "rhizosphere soil" is retained here as distinguished from samples taken entirely outside the root system influence.

Tullgren funnels with soil samples were arranged in series under 40 W light bulbs 19 cm above the soil, maintaining a surface temperature of 35 °C. The funnel stem extended into a 50-ml glass collecting tube standing inside a 1-liter Erlenmeyer flask. The tube contained 10 ml of sterile demineralized water with the stem end of the funnel positioned a few millimeters above the water surface. Alcohol was not used because the animals were needed live for later studies. Each apparatus was wrapped in paper toweling to reduce the amount of water condensation on the glass funnel surfaces. Six of these funnels, each containing 1.1—1.2 kg of composited core samples (depending on soil type) were prepared for each field-plot sampling area of ca. 0.4 ha.

Animals collected in the test tubes of water were decanted through a 5-cm diameter sieve of 100-μm mesh, then refloated by placing the sieve in a shallow dish of water, where total counts were made with a zoom binocular stereomicroscope. Populations were recorded as numbers/g of dry soil and then converted to liters; conversions of grams to liters for each soil type are provided with tables of data.

Field sampling was performed in three major phases. First, collembolan populations were assessed in two differentially fertilized experimental cotton fields of contrasting soil type in April (pre-plant) to indicate the influence of fertilization (and related variables) in the absence of living roots. Secondly, one of these fields was sampled through a growing season primarily to compare rhizosphere with nonrhizosphere populations in nutrient deficient and fertile plots. Finally, rhizosphere effect on populations was determined in cotton fields of three widely separated localities in Alabama.

One of the two experimental fields (Dothan sandy loam), located at Auburn, Alabama, was established by the University Agronomy and Soils Department in 1914 as a long-term rate-of-fertilization study. The field consists of three tiers each divided into plots 6.1 × 29.1 m, which have been subjected to the same fertilizer treatments and a 3-year rotation of cotton, soybeans, and corn for the past 11 years. A mixture of crimson clover (*Trifolium incarnatum* L.) and vetch (*Vicia sativa* L.) follows cotton during the winter months and is turned under in the spring for green manure. A period of fallow is allowed after soybeans [*Glycine max* (L.) MERR.], and winter wheat [*Triticum aestivum* L.] follows corn [*Zea mays* L.]. Fertilization rates[1]) (kg/ha) were: N [NH_4NO_3] ≙ 135, P [P_2O_5] ≙ 33, and K [K_2O] ≙ 83; one-half of the mineral fertilizer was applied just before planting wheat and one-half before cotton. This field also received minor elements and the soil was limed to maintain a *p*H of ca. 5.8—6.5. Foliar sprays were applied to cotton weekly from July 1 (blooming) to September 1 with toxaphene (chlorinated camphene) plus methyl parathion (0,0-dimethyl 0-*p*-nitrophenyl phosphorothionate) at rates of 1.12 + 0.56 kg/ha, respectively. For our study, only the tiers planted to cotton (Auburn 56 cultivar) in 1974 and 1975 were used; the specific plots are shown in Table 1 of the results section.

Another experimental field (Lucedale sandy clay loam) located at Prattville, Alabama consists of plots 6.1 × 26.4 m, each with a different fertilization regime and monocultured to cotton for over

1) Calculation of fertilization rates based on elemental NPK.

40 years; the cotton cultivar at the time of this study was DPL 16, and insecticides applied were as noted above. The five plots chosen for Collembola population assessments are shown in Table 2.

In June of 1975, rhizosphere and nonrhizosphere samples were taken from cotton fields in three areas of Alabama to determine relative populations of Collembola in widely separated fields of different soil type. A fertile plot (NPK + minor elements, Table 1) at the Auburn station served as one of the sample areas; average plant height at sampling time was 27 cm. A small field at the Sand Mountain Substation (De Kalb County) had been in cotton-corn rotation for several years and received the recommended fertilization schedule for that area (NPK as NH_4NO_3, P_2O_5, and K_2O; ≙ 135, 30, and 56 kg/ha). The herbicides trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine) and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] had been applied pre-emergence in April, and the usual insecticide spray schedule was applied at blooming. The soil type was Hartsells fine sandy loam and the cotton (Coker 310) had attained an average height of 25.5 cm at sampling time. The third field, located at the Tennessee Valley Substation in northern Alabama (Limestone County), had been in cotton monoculture for several years and received the recommended fertilization for that area, as noted for Sand Mountain. Trifluralin and the insecticide-acaricide, disulfoton (0,0-diethyl *S*-[2-(ethylthio) ethyl] phosphorodithioate) had been applied pre-plant in April. The soil type was Decatur clay loam and the cotton (Stoneville 213) averaged 30.5 cm in height. All samples were transported to the laboratory in an ice-cooled chest.

Finally, a greenhouse test was conducted to determine the effect of root biomass on populations of Collembola. In a fertile field plot of Dothan sandy loam large cores of soil were taken randomly from cotton rows with a post-hole digger (15 cm diam.) to a depth of 20—25 cm. The cores were composited and mixed thoroughly; mixing was essential in this case so that the collembolan content would be evenly distributed when placed into pots. The soil in 1,100-g quantities was placed in 18 plastic pots with 0.5-cm drainage holes in the bottom; a 1.5-cm layer of autoclaved vermiculite was placed on top of the soil in each pot to prevent drying.

Cotton seed used was Stoneville 213, acid delinted, non-treated seed provided by the Stoneville Pedigreed Seed Co., Stoneville, Mississippi. Twenty seed per pot were planted in twelve pots, and six pots of soil were left unplanted. Populations of Collembola in the soil at time of planting were determined by Tullgren funnel extraction. Three days after emergence, the plants were thinned so that three groups of six pots each contained either no plants, one plant, or four plants per pot. All pots, with or without cotton, were fertilized at weekly intervals with an 8-8-20 formulation. Water was applied according to weight loss.

Six weeks after planting, collembolan populations were determined. Plant tops were cut off at the soil line, and soil plus roots from each six-pot group were composited. Subsamples were placed in six extraction funnels for 7 days. Dry root weights were then calculated by subtracting the dry weights of soil in control pots (no plants) from the weights of soil plus roots in other pots.

All controlled experiments were repeated one or more times. Analysis of variance was performed on the data, and comparison of means made by using Tukey's multiple comparison procedures.

3. Results

3.0. Prefatory note

Two genera of Collembola predominated in cotton fields of Alabama, and only one species of each genus was most frequently collected; these were *Onychiurus encarpatus* DENIS (Poduridae) and *Proisotoma minuta* TULLBERG (Isotomidae). Other collembolans occasionally encountered were omitted from the tabulated results.

Table 1. Relative numbers of Collembola in differentially fertilized plots of Dothan sandy loam at Auburn, Alabama in April prior to planting cotton

Treatment	Variable factor	Winter legume	Average number of Collembola/liter soil		Total
			Onychiurus encarpatus	*Proisotoma minuta*	
O	No fertilizer	—	24.8 b	27.7 d	**52.5** b
PKL	No N	—	365.0 a	16.8 d	**381.8** a
NKL	No P	+	441.0 a	70.4 c	**511.4** a
NPL	No K	+	62.4 b	126.0 a	**188.4** b
NPK	No L	+	51.5 b	20.0 d	**71.5** b
NPKL + m. e.	Complete	+	49.6 b	99.3 b	**148.9** b

N ≙ actual N from NH_4NO_3, P ≙ P_2O_5 K ≙ K_2O, L ≙ lime and m.e. ≙ minor elements. (+) or (—) indicates that plots were or were not planted to a winter cover crop. Numbers of Collembola are average of six 1-liter soil samples per plot; 1 liter of soil dry wt = 1224 g. Values not follwed by a common letter are significantly different ($P < 0.05$).

3.1. Influence of soil fertility

In the long-term fertilization test at Auburn (Dothan sandy loam), sampled in April prior to planting of cotton, highest numbers of *O. encarpatus* and highest total collembolan populations occurred in plots deficient in either N or P (Table 1). *Proisotoma minuta* was more prevalent in K-deficient plots. Total populations in the plot with a history of complete fertilization (NPK + lime and minor elements) were relatively low and not significantly different from those in the unfertilized plot.

The sandy clay loam of the differentially fertilized plots at Prattville, also sampled before planting, yielded very low numbers of collembolans, *P. minuta* being the more prevalent of the two species (Table 2). Highest numbers of this species, and of total collembolans, were extracted from the most fertile plot (NPK ≙ 135 : 30 : 56 kg/ha), and none were found in the unfertilized plot. Specific nutrient elements seemed to have little influence in this case.

3.2. Influence of soil fertility and rhizosphere

The data in Table 3 represent a series of comparative samplings through the growing season of one year and into the early part of the next season, designed primarily to show rhizosphere effect. The pre-emergence samplings (no rhizosphere) in April, 1974, showed significantly higher numbers of both species and of total collembolans in the fertile plot than in unfertilized soil. The first rhizosphere sampling in May, when plants were approximately 15 cm high, revealed even greater differences in populations between the contrasting fertility treatments. Over 20 times more total animals were extracted from the rhizosphere of completely fertilized plants.

The June, 1974 samplings (Table 3) featured a comparison of rhizosphere vs. non-rhizosphere populations of the fertile plot only. Numbers of both genera were many times greater in rhizosphere than in soil 20 cm distant from the tap root. The August, 1974 samplings again compared only rhizosphere populations of the two plots and continued to reveal the contrasting fertility influence as plants were approaching maturity. In the following month of September this effect still persisted in mature plants.

A final sampling in June of the next growing season (Table 3, bottom) compared rhizosphere with nonrhizosphere populations in the two plots. The results continued to show clearly the beneficial effect of rhizosphere on numbers of Collembola and the unfavorable effect of soil that is deficient in the major inorganic elements.

3.3. Rhizosphere effect in three localities

The three widely separated cotton fields in Alabama were sampled in June, 1975. Large differences between rhizosphere (R) and nonrhizosphere (S) populations for both genera

Table 2. Relative numbers of Collembola in differentially fertilized plots of Lucedale sandy clay loam at Prattville, Alabama in April prior to planting cotton

Treatment (NPK)	Variable factor	Average number of Collembola/ liter soil		Total
		Onychiurus encarpatus	*Proisotoma minuta*	
0— 0— 0	No fertilizer	0.0	0.0 c	**0.0** c
0—30—56	No N	0.0	12.2 bc	**12.2** bc
135— 0—56	No P	0.4	23.7 b	**24.1** b
135—30— 0	No K	2.1	15.3 bc	**17.4** bc
135—30—56	Complete	0.0	77.1 a	**77.1** a

N ≙ actual nitrogen from NH_4NO_3, P ≙ elemental phosphorus from P_2O_5, and K ≙ elemental potasium from K_2O in kg/ha. Numbers of Collembola are averages of six 1-liter soil samples per plot; 1 liter dry wt = 1266 g.

Values not followed by a common letter are significantly different ($P < 0.05$).

Table 3. Populations of Collembola in fertile and nutrient-deficient cotton plots of Dothan sandy loam, determined during the growing season

	Treatment	Kind of sample	Average number of Collembola/liter soil		Total
			Onychiurus encarpatus	*Proisotoma minuta*	
April, 1974	0	Preemergence	25.2	67.2	**92.4**
	NPKL + m.e.	Preemergence	109.2	113.4	**222.6**
May	0	Rhizosphere	8.4	2.1	**10.5**
	NPKL + m.e.	Rhizosphere	86.1	140.7	**226.8**
June	NPKL + m.e.	Rhizosphere	94.5	144.9	**239.4**
	NPKL + m.e.	Nonrhizosphere	14.7	21.0	**35.7**
August	0	Rhizosphere	31.5	16.8	**48.3**
	NPKL + m.e.	Rhizosphere	90.3	142.8	**233.1**
September	0	Rhizosphere	27.3	71.4	**98.7**
	NPKL + m.e.	Rhizosphere	111.3	121.8	**233.1**
June, 1975	0	Rhizosphere	20.4	26.9	**47.3**
	0	Nonrhizosphere	8.0	7.8	**15.8**
	NPKL + m.e.	Rhizosphere	143.9	106.1	**250.0**
	NPKL + m.e.	Nonrhizosphere	35.7	56.1	**91.8**

N ≙ actual nitrogen from NH_4NO_3, P ≙ P_2O_5, K ≙ K_2O, L ≙ lime, and m.e. = minor elements. Numbers of Collembola are average of six 1-liter samples of soil per plot; 1 liter dry wt = 1224 g. Differences between treatments for each sampling period were significant ($P < 0.01$).

Table 4. Populatinos of Collembola in three widely separated Alabama cotton fields, determined in June

Field	Soil type	Average number of Collembola/liter soil						
		Onychiurus encarpatus		*Proisotoma minuta*		Total		
		R	S	R	S	R	S	R/S
Auburn	Dothan sandy loam	143.9	35.7	106.1	56.1	**50.0**	**91.8**	2.7
Sand Mountain	Hartsells f. sandy loam	14.1	13.2	131.7	58.2	**154.8**	**71.4**	2.0
Tennessee Valley	Decatur clay	1.7	1.5	6.7	6.3	**8.4**	**7.8**	1.1

Numbers are average of six 1-liter samples taken from each composite field sample; conversinos to dry wt/liter are: Dothan s. l. 1224 g, Hartsells f. s. l. 1238 g, and Decatur c. l. 1275 g. R = rhizosphere; S = nonrhizosphere, soil distant from the root system. Rhizosphere vs nonrhizosphere for *O. encarpatus* at Sand Mountain, and for both species at Tennessee Valley not significantly different ($P < 0.05$).

Table 5. Effect of increasing cotton root biomass on populations of Collembola in greenhouse pots of Dothan sandy loam, determined 44 days after planting

Number of plants/pot	Root weight (g)	Average number of Collembola/liter soil
0	0.0	95.6
1	7.8	253.5
4	31.5	377.4

Root-weight values and numbers of Collembola are averages of six pots. All compared values were significantly different ($P < 0.01$).

of collembolans were found in the sandy loam at Auburn (Table 4). The R/S ratio value for total populations was 2.7. In the fine sandy loam of the Sand Mountain area in northeast Alabama, only *P. minuta* was significantly more abundant in the rhizosphere, but the total number of collembolans was greater in the rhizosphere, reflecting an R/S value of 2.0. Populations in the clay loam of the Tennessee Valley area were very low and differences between R and S for either species were not statistically different.

3.4. Effect of cotton-root biomass on populations

The influence of cotton-root biomass on the biuld-up of collembolan populations can be clearly seen in Table 5. After 44 days of plant growth the collembolan population associated with a single-plant root system had increased to a value nearly $2^1/_2$ times that in soil without roots; numbers in soil with four plants increased to nearly 4 times the number in plant-free soil and $1^1/_2$ times the number in pots with one plant.

4. Discussion

The sampling and extraction procedures used in this investigation are considered to be quite adequate for the objectives, though it is recognized that soil-organism populations determined by most methods are only relative. Our soil-core samples collected a mixture of rhizosphere and nonrhizosphere soil, but the mixture reflected a distinct influence of the root system as distinguished from soil entirely free of roots; thus we retained the term "rhizosphere" for convenience of this comparison. It is reasonable to believe that some collembolans were lost or destroyed during sampling and transportation. However, from the time of extraction-funnel preparation to the end of the 7-day collection period, extraction of living animals was found to be highly efficient (95 %) as determined by soil infestation and recovery tests. The relatively low populations in these cultivated fields were expected in view of the known influence of cultural practices on microarthropods (WALLWORK 1976); crop rotation and monoculture in particular tend to reduce species diversity. This was evident in our study, which yielded predominantly only two species.

In the spring, prior to planting cotton, total numbers of *O. encarpatus* and *P. minuta* were higher in a sandy loam deficient in either N or P than in completely fertilized soil or in plots never fertilized. The sandy clay loam at Prattville (Tab. 2), though yielding lower total numbers of animals, showed the expected favorable influence of high fertility. The contrast in results from these two areas probably was related more to time of supplementary fertilization than to soil type or other factors. In the Auburn plots, one-half of the fertilizer was applied just before planting cotton and one-half before wheat in the 3-year rotation period; the Prattville plots, under cotton monoculture, were fertilized each year at planting time. Neither area had been fertilized for the current season at the time of the first samplings. More convincing evidence of a favorable fertility influence on collembolan populations was obtained from samplings begun at fertilization and planting time and followed through a growing season (Table 3).

Collembola are generally more abundant in soil of high organic matter content, which one would expect to find resulting from a complete fertilization regime that favors plant growth. Though supplemental inorganic fertilization also may increase the organic matter decomposition process, this would be accompanied by increased populations of the microflora, a potential food source for Collembola. EDWARDS and LOFTY (1969) have shown clearly the favorable influence of organic fertilization on collembolan activities. Our study was not designed to clarify specific fertilization effects and did not do so; however, it is interesting, and perhaps logical, to speculate that fluctuating collembolan numbers are related to specific nutrient-element effects on the quality or quantity of the soil microflora available as a food source. Both species of animals featured in this study have chewing mouth parts and the capability of feeding on fungi (WIGGINS 1976).

The collembolans were consistently more abundant in cotton rhizosphere than 20 cm distant from root influence in both fertile and nutrient-deficient soil. Though an explanation for higher populations in the root zone was not derived experimentally in this study, conjecture could be made regarding the probable indirect effects of root exudates on the fauna. Exudate stimulation of the microflora in the rhizosphere is well known, and these fungal feeding collembolans would be expected to benefit indirectly. These animals also seek areas of moisture and they retreat from areas of water deficit. Though root systems extract moisture from soil, water moving to the root surface coupled with exudation from the roots presumably provides a favorable environment for Collembola.

Many references have been made to the actual and probable interactions between the fauna and microflora (KEVAN 1965; BUTCHER, SNIDER, and SNIDER 1971), relating for the most part to their dual role in the humification of organic matter and in soil formation. In this regard, KEVAN points out that there is an inseparable association between the soil fauna and microflora. It is just as likely that this association is even more intense at the root surface, though no specific data are available on the interactions between components of the microflora and fauna in the rhizosphere of cotton. It is a challenging thought to reflect on the possible role of mycofagous Collembola in the competitive microbial colonization of the root zone and, subsequently, their influence on plant pathogen activity. Our present ongoing investigation is designed to provide such information.

5. Summary · Zusammenfassung

Investigations in rhizosphere ecology cannot be complete without knowledge of the fauna as well as the microflora. Two species of Collembola, *Onychiurus encarpatus* and *Proisotoma minuta*, were predominant in cotton fields in Alabama through a growing season. Supplementary fertilization induced higher numbers, and populations were significantly greater at all times in rhizosphere soil than 20 cm distant from the root zone. A direct relation was shown between increasing cotton-root biomass and populations of Collembola. It is suggested that fungal and bacterial feeding collembolans may have a major role in determining the competitive interactions among components of the microflora at the root surface.

Einfluß der Bodenfruchtbarkeit und der Rhizosphäre von Baumwollpflanzen auf den Collembolenbesatz

Zur ökologischen Erforschung der Rhizosphäre sind Kenntnisse der Bodenfauna und der Bodenmikroflora nötig. Auf den während einer Vegetationsperiode untersuchten Baumwollfeldern in Alabama herrschten zwei Collembolenarten, *Onychiurus encarpatus* und *Proisotoma minuta*, vor. Zusätzliche Düngung führte zu einem Anstieg der Abundanz der Collembolen und zu jeder Zeit war die Besatzdichte in der Rhizosphäre höher als in 20 cm Entfernung von der Wurzelzone. Es wurde eine direkte Beziehung zwischen der zunehmenden Wurzelmasse der Baumwollpflanzen und der Besatzdichte der Collembolen gezeigt. Es wird vermutet, daß pilz- und bakterienfressende Colembolen bei der Prägung der kompetitiven Interaktionen zwischen den Elementen der Rhizosphären-Mikroflora eine größere Rolle spielen.

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7. Literature

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